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Evaluation of Environmental Concentrations of 4-Nonylphenol using GC-MS

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Appendix of Headings and Subheading

Appendix of Tables and Figures

Introduction:

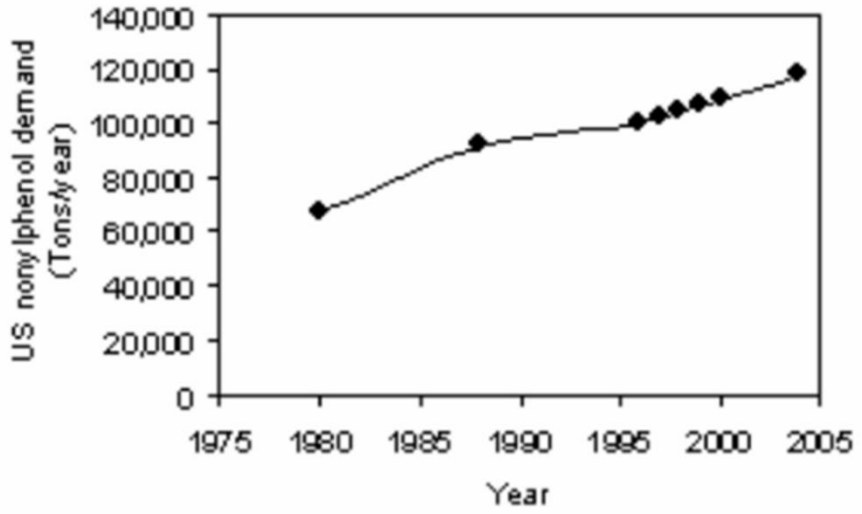
With the continual growth of global industry, there are a large amount of synthetic compounds being introduced into the environment. [1] Testing the possible effects of these compounds are a concern for various environmental agencies. One category of compounds widely investigated are endocrine disruptors. Endocrine disruptors are chemical compounds that disrupt an organism's endogenous hormone signaling pathway upon introduction to an organism.[2] The effect of these compounds on an organism's system at bioactive concentrations, are developmental malformation, interference with reproduction, increased cancer risk, and disturbances in the immune and nervous system function.[3] The consequences of acute exposure can be severe. The molecular mechanism at which disruption occurs is still not widely agreed upon due to the sheer number of compounds existing with disruptive capabilities. One such compound with endogenous disruptive potential is 4-nonylphenol (NP), a compound hypothesized to be found in Central California.

Central California is one of the most highly farmed regions in the country, earning its nick name as the Fertile Crescent of California or the California Central Valley. This area of land has a significant geographic structure, a lower elevation than the surrounding area and is pressed relatively close to the Sierra Nevada mountain range. The California Central Valley is estimated to cover 20,000 square miles and is primarily farmland. This highly fertile portion of the state of California is responsible for supplying the country with approximately 40% of its fruits, nuts, and table foods. [4] The high crop yield of this area requires the usage of pesticides. It is estimated that in 2008 alone, 516 million pounds of pesticides were used for US agriculture. [5] Due to the high content of ethoxylates, such as nonylphenol ethoxylates (NPE) in

pesticides, it is expected that in areas of high agricultural density, there are also large amounts of nonylphenol ethoxylates or nonylphenol (NP) being introduced into the environment. The study of how NP interacts within an ecosystem is imperative since nonylphenol ethoxylate (NPE) comprise 80% of all alkylphenol ethoxylates compounds produced globally. [6-7]

NP is produced from NPEs as an aerobic bacterial breakdown product as well as through photolysis, and it is both NP and NPEs that are used in many industrial products. [7-8] NPEs are largely used in both household and industrial products such as detergents, surfactant, an emulsifier, and formulant making up to 10% of pesticides by weight. [9-11]. The demand, specifically in the US, has increased linearly, as shown in figure 1.

FIGURE 1: Figure from Vasquez et al. showing the trend of demand of products containing nonylphenol by year per tons/year. [12]



Due to the demand for products containing or requiring NP for production in recent years, [13] the potential for exposure has increased globally. The increased concentration of NP in the environment has posed many issues due to the compound's high lipophilicity, high $\log K_{ow}$ at 5.73, and low solubility in water (under 7ppm) [14]. A organism can be exposed to the compound through any of the many transport routes, for example: oral, respiratory or

precutaneous, [15] NP bioaccumulate in the nonpolar fatty tissues of the body and affect organs such as the kidneys, the liver, and those associated with reproductive function. [16] The introduction of acute concentrations of NP has been shown to cause damage to the respiratory track, eyes, and skin, while greater concentrations have an effect on the reproductive system of the organism. [17] As well as being lipophilic, NP is also a xenoestrogen, mimicking the structure of 17 β-estradiol (as seen in figure 2), allowing it to bind to estrogen receptors and compete for binding with estradiol.

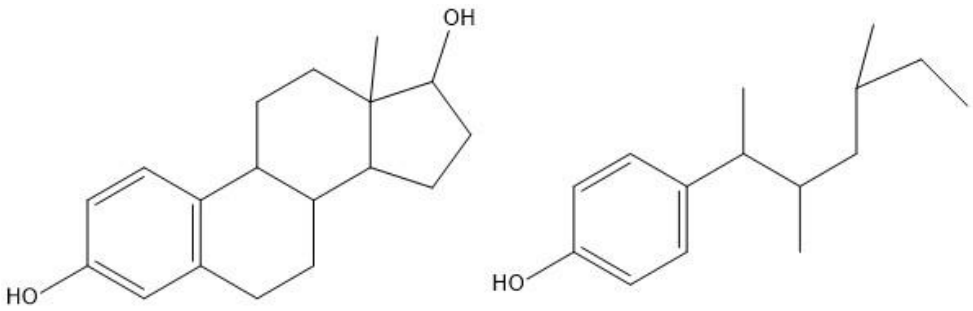


Figure 2: Structures of 17 β –Estradiol and estrogenically active isomer of 4-nonylphenol. Isomer rotated to show similarities between structures.

Nonylphenol has been shown to causes hormonal disruption at biologically active concentrations in the environment at levels of 10,000 ug/L and influences the development and maintenance of female secondary sexual characteristics in organisms as well as the maturation rate of sex organs in juveniles. [18] NP stimulates the expression of the hormonal genes for vitellogenin and estrogen, causing the decrease in androgen production, ultimately inducing hyper-feminization and infertility. 4NP is capable of interfering with the endocrine system at exposure concentrations as low as 0.01 mg/day in rodents. [17] NP is capable of triggering respiratory toxicity in cells associated with a change in mitochondrial membrane permeability, disrupting the active chain transport of calcium in muscular sarcoplasmic reticulum. This

disruption of active chain transport in turn alters the growth and differentiation of neural stem cells, as well as the induction of apoptosis of these cells, inducing the production of telemetric associations and chromosomal aberrations ultimately triggering the alteration of cell cycle kinetics. [18]

NP is a carcinogen as well. Recent studies linking high concentrations of the compound in mice with increased mammary gland development, acting as a possible promoter for estrogen dependent cancers such as breast or ovarian. [20-22] This influence on estrogen dependent cancers is due to the estrogen receptor mediated mechanism, 4NP has the ability to interrupt normal mammary gland differentiation at high concentration. [19]

Currently, very little is known as to the effects of long term exposure to NP in humans specifically. Additionally, the effect of this compound on a human system must be extrapolated based on the previously described effects the compound has on animal test subjects. The bodily concentrations of NP needed for bioactivation in humans is expected to be much larger than that needed for acute toxicity in smaller research animals, humans having a much smaller surface area to mass ratio. [1] Human exposure to nonylphenol is much harder to quantify than in animal studies due to the many uncontrolled variables for exposure. It is also difficult to trace human NP exposure to one environmental source as there are various methods of introduction of the compound to the human system. Consumption of contaminated water, crops milk, meat, or through occupational exposure are some of the more common ways in which nonylphenol is introduced into the human system. [18] Based on concentrations in food and consumption rates, the average adult daily intake is anywhere between 0.067 and 0.370 µg/kg of body weight/day. [8] The maximum acceptable concentration of environmental aqueous nonylphenol, based on

acute toxicity of aquatic organisms, is around 10 µg/L, while the EPA describes ambient water to be safe for exposure if the nonylphenol concentration is less than 6.6 µg/L. [18] Because of NPs bio-accumulative properties, once it is introduced to that environmental or human system, it remains there until excreted or flushed. Only 10% of the bodily accumulation of nonylphenol is flushed via urine or feces. [18]

Nonylphenol has been found in water samples in many places around the globe, demonstrating the ubiquity of NP and NPEs in the environment. The range of NP concentrations found in previous research was be from non-detectable levels to that of several micrograms per liter in aqueous samples. [22] These studies all focused on the determination of NP in surface water using differing methods of analysis. To determine the concentration of NP in a southern Chinese river, one study, performed by Zhao et al., made use of solid phase extraction to derivatize the sample. The samples were then run on a GC-MS making use of a chemical ionization source. The highest concentration measured was 11.3 µg/L in the Shijing River [23]. Klontza et al. used a similar method of sample preparation for aqueous nonylphenol containing samples. Solid phase extraction was used and NP concentrations were measured using GC-MS. In this study, water samples were collected from different stages of municipal water treatment in China. Overall, the concentration of NP in the raw, untreated waste water was measured at 7.92 µg/L. [24] It is important to note that the study of environmental NP exposure is a popular subject for study in China as recently the country began to regulate the compound.

Several studies have also been conducted in the United States as well. A study performed by Brown et al. measuring the concentration of NP in different aquatic ecosystems around the Midwestern of the United States. Water samples were prepared via liquid phase

extraction, using methylene chloride as an extraction solvent, and analyzed using GC-MS. Brown et al. found that sampling location made a difference in the concentration measured, indicating that the degree at which an environment is susceptible to NP exposure is spatially dependent. Concentrations of NP ranged from 0.9 to 2.5 μg/L, approximately three times smaller than what was found in the previous Chinese studies. [25]

There is currently a lack of research quantifying the concentration of nonylphenol in the body. It may be possible to look at environmental concentrations of alkylphenolic compounds to make an estimate as to the concentration of that compound that may be found in an organism's system. This possible relationship between the environmental concentration of a compound and that which may be found in the body, is based on the idea of how an organism interact with different portions of the environment. To study this interaction, we can use the theory of environmental compartmentalization. This is the theory that varying concentrations of an analyte can be found in different portions of the environment based on the relative chemical properties of the compound of interest. This form of environmental analysis is useful for understanding the potential of NP contamination in both environmental and biological systems. In the case of nonylphenol, higher concentration of the compound has been found in the soil and sediment portion of the environment as opposed to the air or water compartment based on the high log Kow value of the compound. This is mainly due to the hydrophobicity of the compound and its high octanol to water partition coefficient. For this reason, it much easier to quantify the concentration of this compound in this compartment; however, if you wanted to quantify concentrations of NP in the air or water compartment, it is essential to use more sophisticated forms of instrumentation or sampling methods.

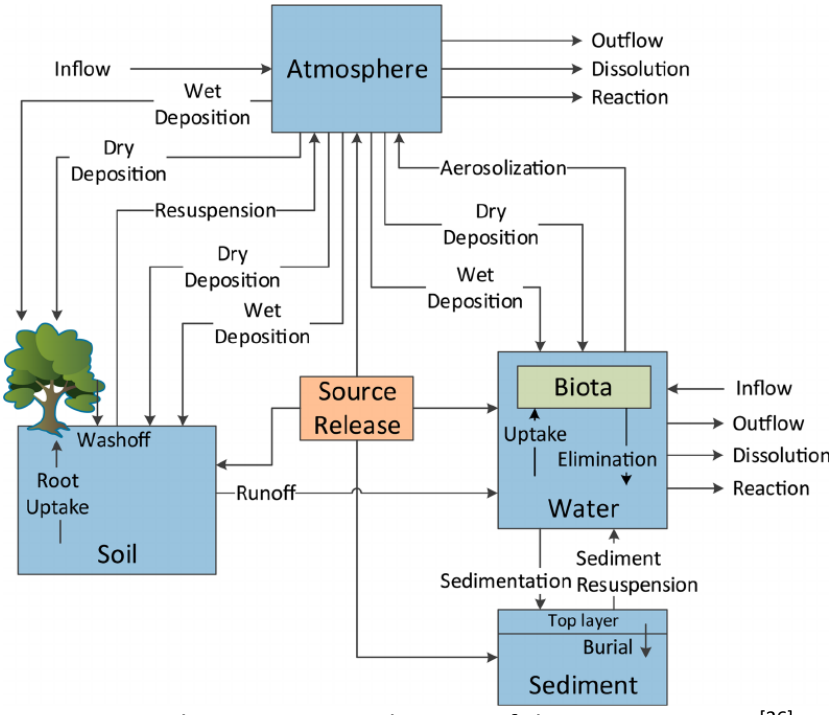


Figure 3: Simple environmental compartmentalization of the environment. [26]

Of the many sampling techniques available, there are four main ways in which environmental samples are obtained with regards to the air and water portions of the environment for the detection NP: grab, passive, assisted, or automated/continuous sampling. The most widely used environmental sampling technique is the grab sample. A grab sample provides a snap shot like view of the sample location at the time and point of sampling and is a useful method of sample collection as it has a low cost and skill associated with its usage and is widely available.

The sensitivity of a grab sampling is entirely dependent on the collection of a sample with the analyte present and multiple samples can be obtained to determine the variability in analyte concentration at the sampling site. [27] Regarding aqueous nonylphenol sampling, this technique can in some ways be equated to finding a needle in a hay stack in terms of analyte detection, since nonylphenol has a high $\log K_{ow}$ and tends to partition to non-polar substances.

This makes the obtaining a high concentration NP sample difficult. NP doesn't preferentially want to stay in an aqueous medium and Samples must be obtained in polar receptacles to prevent the binding of the compound to the container. Another widely used sampling technique is passive sampling. This sample technique also has a low cost and skill associated with sample collection; however, much like a grab sample, this technique is not event specific and depends on the obtainment of a sample at a specific time. This technique is generally used for the collection of aerosols or gas phase analytes as well as aqueous sampling and allows for the determination of a time dependent average regarding concentration and or deposition rate. One example of a passive sampling apparatus is a Passive In-Situ Concentration Extraction Sampler (PISCES). This method of sample collection relies on diffusion to accumulate analyte in the sampler. [28] Much like a grab sample, the collection of your analyte with this technique is also dependent on different parameters such as particulate deposition rate or sample location. The other two sampling techniques, assisted and automated sampling, are not as widely used due to their high skill, low availability and high cost.

Assisted sampling allows for the collection of environmental samples not always readily accessible or feasible for collection by researchers. One examples of a method of assisted sampling is the usage of unmanned aerial vehicles for the obtainment of samples. Generally, assisted sampling methods are more expensive than passive or grab sampling and requires more skill for operation. [29]

Automated samplers are those that can be set up prior to the time of sampling and programmed to obtain a sample based on factors such as time, in-stream flow, or water level. With this technology, it is possible to continuously monitor a system. One example of an

automated water sampler is an ISCO 3700. This instrument allows for the collection of composite or sequential samples based on flow rate, time, or storm condition. [30] This method of sampling however is more expensive than the previous methods of sampling however it allows for the collection of samples with greater precision and autonomy.[27]

When deciding to use one of these four major forms of sampling, it is important to consider the characteristics associated with each technique, as shown in figure 4. Due to the low cost and skill associated with both grab and passive sample collection, these two methods are widely used in low budget environmental work. Unfortunately, these techniques tend to have a low sensitive associated with sample collection. One way to compensate for the usage of non-sensitive sampling techniques is to make use of highly sophisticated instruments for analysis with lower limits of detection.

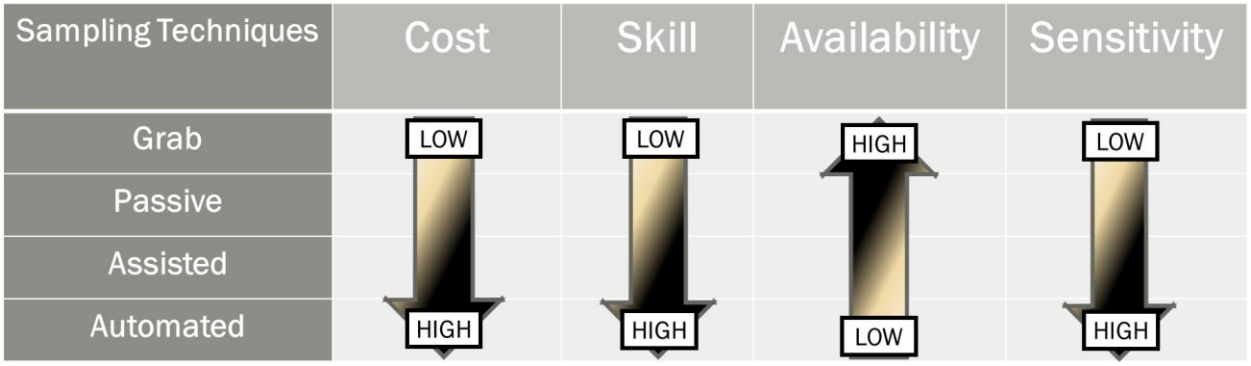


Figure 4: Most prevalent methods of environmental sampling for the detection of analyte in both the air and water compartments of the environment. Gradient arrows indicate the range from high to low density for each category of consideration.

Gas Chromatography Mass Spectrometry (GC-MS) is one such form of instrumentation used for the detection of alkylphenols in an aqueous environment. GC-MS has a low detection limit and is highly sensitivity. Chromatography is a means of matrix separation and has been shown to be useful for the detection of alkyphenolic compounds. Separation occurs in the gas

phase based on the polarity of the solution through an open capillary column. The more similar the polarity of the analyte is to the polarity of the sorbent, the better the attractive force between the two, allowing for better separation of the analyte from the solution. After separation occurs, the solution is ionized via electron bombardment, producing mass fragments with varying m/z ratios. Two of the most sensitive forms of mass analysis are ion trap and time of flight. For time of flight analysis, the charged analyte particles then enter an electric field causing the ions to all travel at the same relative energy towards a charged detector. Smaller ions will travel faster to the detector plate due to the velocity of travel depending on the mass to change ratio of the particles. This differential velocity causes ion sorting based solely on mass and change. Different intensities with ions of different masses are observed at different times at the detector. From these different mass/charge ratios (given in intensity), the concentration of the analyte and its structure can be determined. [31] For ion trap mass spectrometric analysis, emerging compounds are eluted into the ion trap mass spectrophotometer through a heated transfer line. The ions, once introduced to the ion trap cavity, being to circulate in stable three dimensional orbits due to the applied radio frequency voltages applied to the central ring. Changing the applied voltage causes the destabilization of the component's orbits around the ion trap cavity, allowing for the analysis of the mass fragments. [24]

The goal of this study is to detect and quantify environmental concentrations of 4-nonylphenol and investigate spatial trends in concentration. For the quantification of NP concentration in the environment, environmental samples were obtained either through the collection of atmospheric deposition or watershed grab samples. The concentration of nonylphenol in the environmental samples were then determined using gas chromatography

mass spectrometry. Correlations between NP concentrations and environmental factors such as topography, wind, population density, agricultural density, and geographic features were investigated using ArcGIS analysis.

The goal of this project is to investigate the correlation between the occurrence of NP in the environment and various environmental factors. From an ecotoxicology standpoint, we seek to understand how human produced artificial compounds are effecting the health of not only the environment but also humans directly and this is a possible avenue for future study. To successfully correlate these two variables would allow for further study into how NP may be partitioning into other systems, like the human body, and may ultimately lead to the enactment of laws limiting the usage of NP and NPE derivatives in agriculture.

Methods

Sampling Methods

Sample locations were determined based on each sites susceptibility and risk to exposure to nonylphenol.

Grab samples were obtained from the shoreline of each location during the month of July 2017. Specific sample locations were determined based on qualitative observations of the sample site as well as with the attempt of representative sampling, as shown in figures 6-10. Water samples were collected 1L water samples were collected in amber glass bottles with foil lined caps with gloved hands at a depth of approximately 3 feet. Sample containers were completely filled to eliminate empty possible air interaction. The samples were taken in

duplicate and acid shocked using approximately 10 mL of 6 M H_2SO_4. The samples were kept on ice while transported. The samples were stored at 4°C out of direct light until processed.

Passive atmospheric samplers were used to collect deposition samples about the University of Redlands as aquatic conditions were not available for grab sampling. Passive atmospheric samples were collected via an aluminum funnel containing a mesh screen fastened to an amber glass container. Both the funnel and glass receptacle were fastened together. Sample locations were determined based on lowest potential of anthropogenic influence. Samples were left in unshaded locations for one month's time.

Figure 5: Schematic of passive atmospheric sampler. The yellow disk within the sampling funnel is a filter to remove crude materials from being collected in the collection flask. The brown container below the sampling funnel is the amber glass collection vial.

Organic Extraction and Sample Preparation

500 mL of each water sample were added to a 1.000 L separatory funnel. Each portion of lake water was extracted 3 times using 100 mL of a 1:1 Hexane-acetone solution. Only samples visibly opaque or containing solid were filtered before extraction. Vacuum filtration was performed using a mobile phase filter and 0.45 µm filter paper. The solvent was then extracted from the sample using a Kuderna Danish solvent extractor, leaving approximately 4-6 mL. The

samples were then condensed under nitrogen gas to a final volume of 1 mL. Approximately 1 µL of sample was then injected into the GC-MS.

GC-MS Method Specifications

Instrument was run in total ion monitoring mode (TIM). Injector temperature program set from 60-360°C at 100°C/min and held at 360°C for 20 minutes. The column oven was programmed to start at from 80-390°C at 15°C/min and held at 390°C for 5 minutes. The detector temperature was set to 400°C with hydrogen and nitrogen gas for flow. Helium gas was used as a carrier gas with a flow rate of 50 cm/s. The injection volume was set to 0.5 – 1.0 µL.

The mass spectrometer (MS) was operated in EI mode using an ionization energy of 70 eV and the ion source temperature was set to 200°C. The MS injector column was set to 150°C, with an injection volume of 0.5 – 1.0 µL. and the column oven programmed from 80-350°C at 15°C/min at 15°C/min and held at 350°C for 5 minutes. Helium gas was used as a carrier gas for the MS with a flow rate of 50 cm/s.

Sample Locations

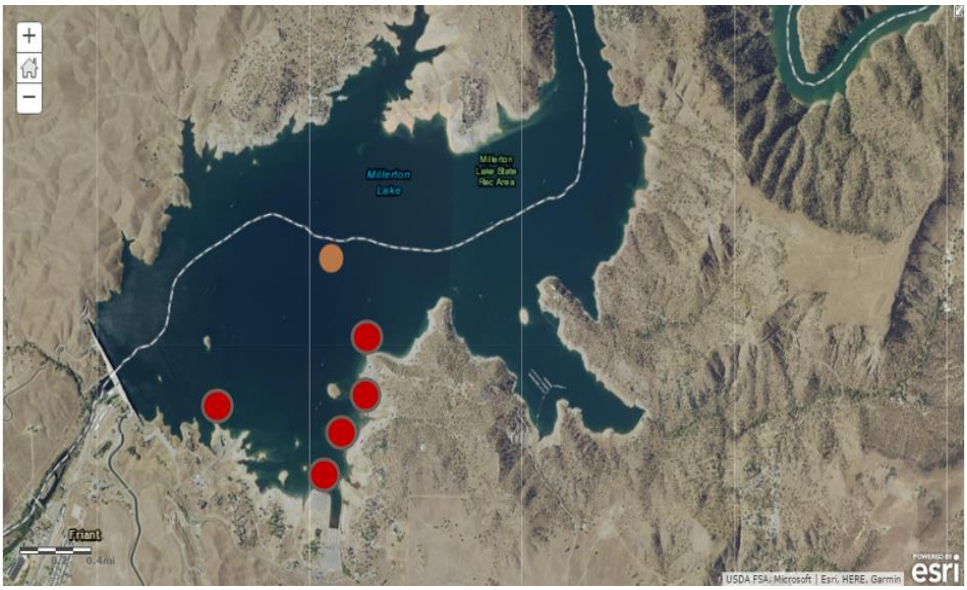


Figure 6: Gis map showing sampling location about Lake Millerton in Fresno, CA. Grab samples were taken along the southern shore of the manmade lake Sample locations are indicated by the red circles. The orange marker indicates the overall site of study.

Figure 7: Gis map showing sampling location about Lake Perris in Perris, CA. Grab samples were taken along the northern shore of the manmade lake and at the central point of the lake. Sample locations are indicated by the red circles.

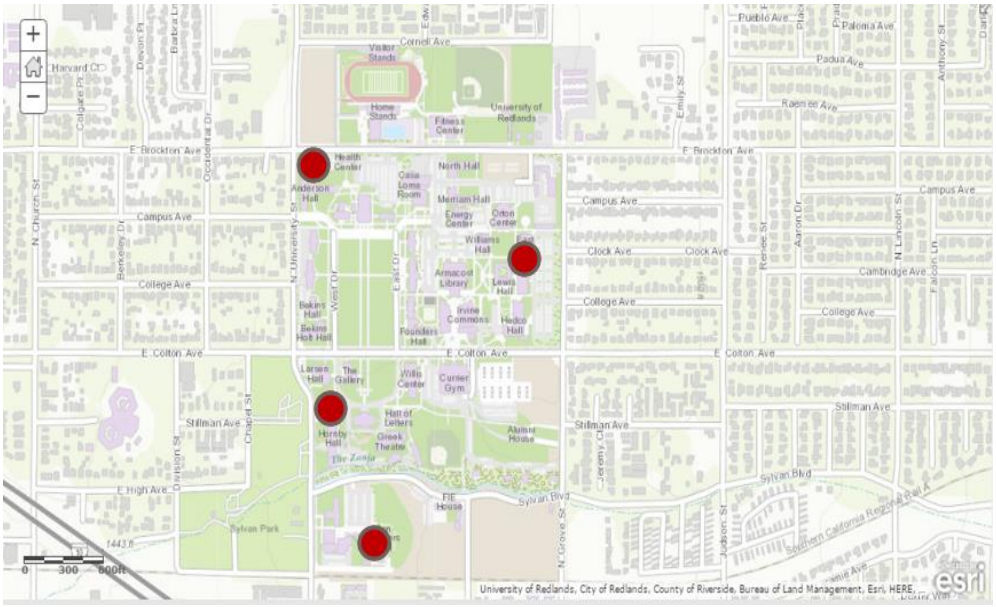


Figure 8: Gis map showing sampling location about the University of Redlands in Redlands, CA. Red dots on the map indicate the location of passive atmospheric deposition collectors.

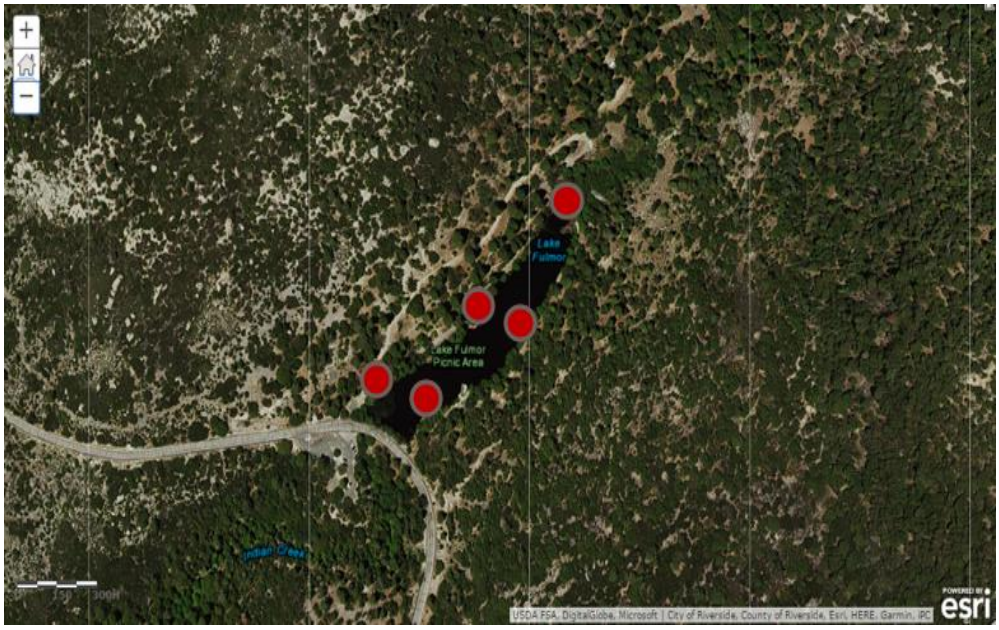


Figure 9: Gis map showing sampling location about Lake Fulmor in Idyllwild, CA. Grab samples were collected around the lake, locations indicated by the red dots.

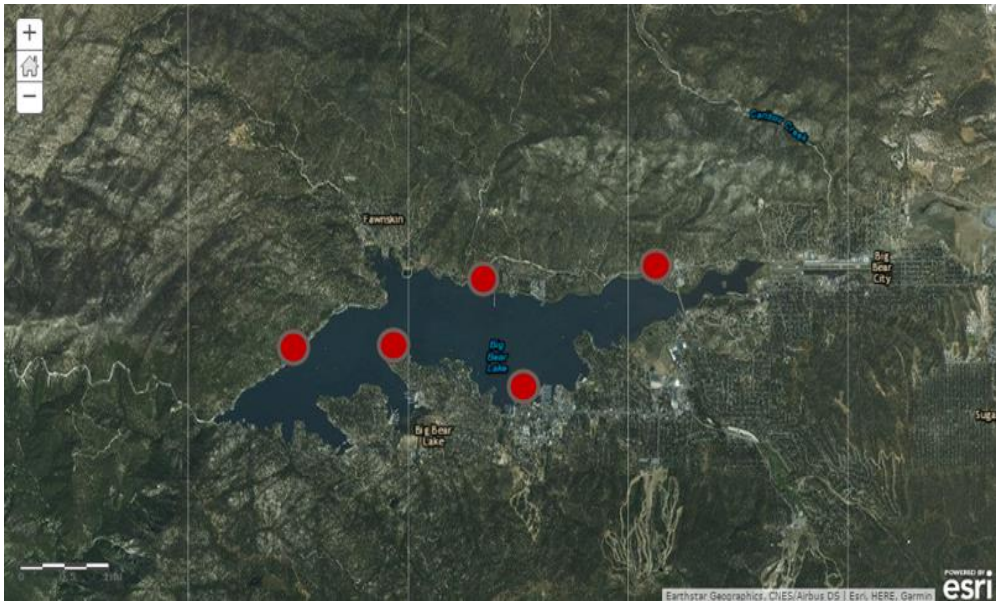


Figure 10: Gis map showing sampling location about Big Bear Lake in Big Bear, CA. Grab samples were collected around the lake at various locations as indicated by the red circles.

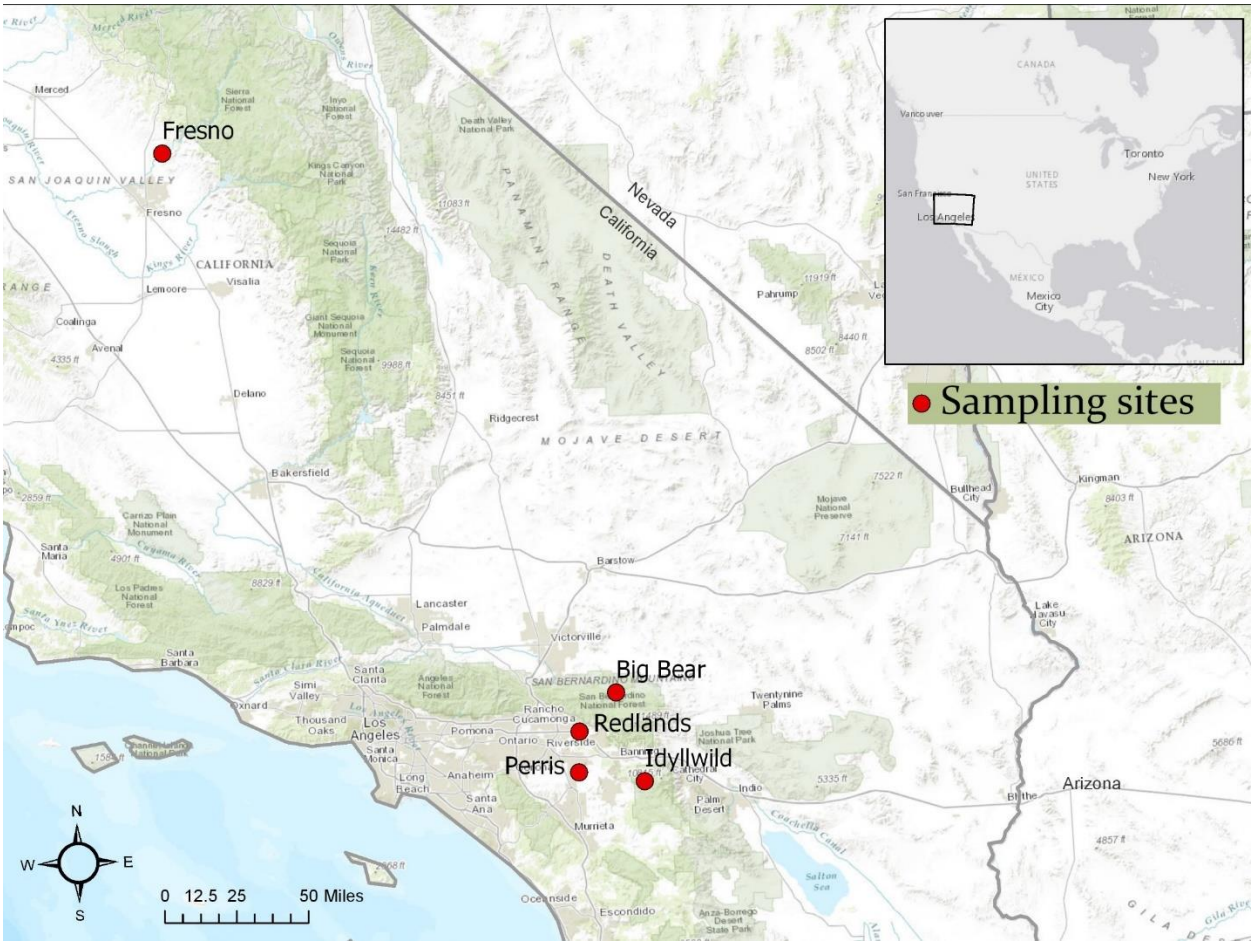


Figure 11: ArcGIS Pro map showing sampling site chosen for this study. Each location represents the location of sampling and the map is a satellite image of the west coast. Obtained 1/28/17.

The method detection limit for the analysis of NP using GC-MS was determined in two different manners. First the theoretical method detection limit (Ydl) was calculated using the equation:

$$Ydl = \frac{3s}{m}$$

This method takes into account both the standard deviation of the blank sample baseline (s) divided by the slope of the standard curve (m) and is specific to the method of analysis. This method of detection limit is accurate; however, with chromatography, the detection limit it calculates is more theoretical in nature and there are other factors must be considered in method detection limit determination. A more practical method detection limit was measured by determining the lowest concentration of analyte reliably and consistently observed. This was performed by taking lower and lower concentration of analyte until a consistence signal was no longer observable.

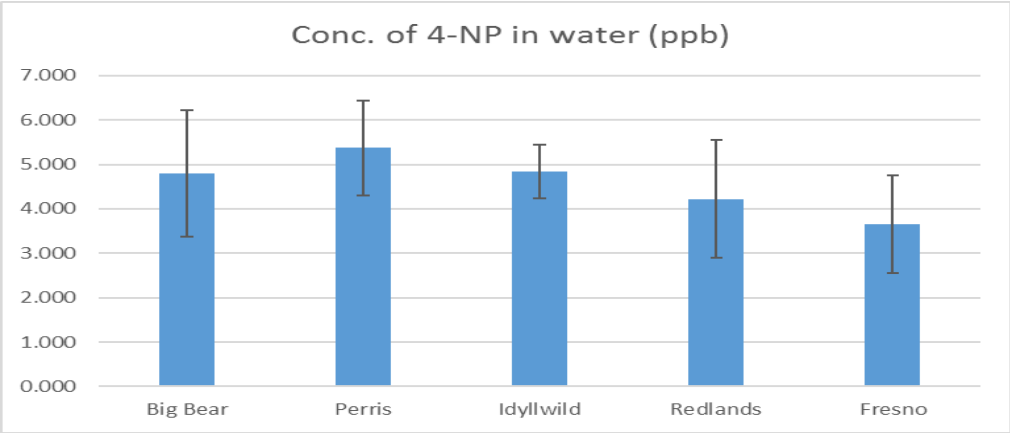
Results & Discussion

Determination of environmental concentrations of NP were performed using GC-MS. A standard curve was produced to measure the concentration of nonylphenol in each sample. Using the Linear regression equation form the standard curve produced, the concentration of NP in each sample was determined.

Table 1: Average concentrations and standard deviations of NP using GC-MS for each sample location measured. Concentration and standard deviation given in ppb or µg/L, while standard deviation is measured using all individual measurements for each respective sampling location excluding those non-detectable. N=4.

GCMS			
	City	Conc. of 4-NP in water (µg/L)	Standard Deviation (µg/L)
	Big Bear	4.79	1.42
	Perris	5.37	1.07
	Idyllwild	4.84	0.61
	Redlands	4.22	1.32
	Fresno	3.66	1.10

Figure 12: Bar graph showing the concentration of 4-NP using GC-MS with respect to sample site. Error bars show standard deviation in data for each site. N=4.



Based on GC analysis, the concentrations shown in figure 12 were determined. For the analysis of NP using GC-MS, the detection limit was calculated to be between 0.500 mg/L and 0.750 mg/L, confirming the calculated theoretical method detection limit (0.666 mg/L). The signal threshold used for the determination of the method detection limit was 0.8×10^2 intensity.

Initially, the concentration range of the sampling sites were predicted based on the relative distance from agricultural density centers. This hypothesis was based on the fact that since NPEs make-up 10% by weight of pesticides, wherever there is an abundance of pesticide usage, we hypothetically should also be able to measure a large concentration of NP being introduced into the environment.

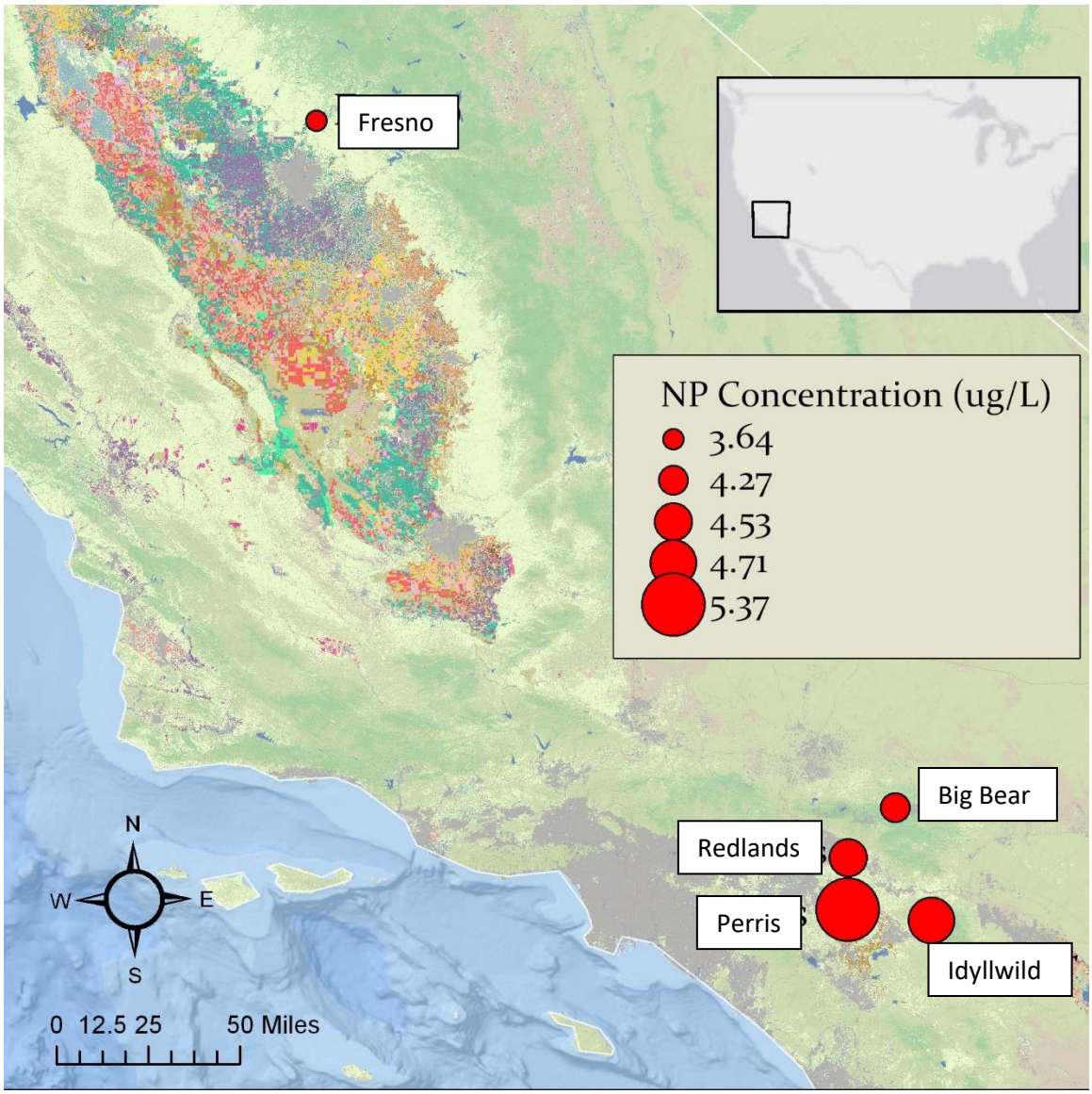


Figure 13: ArcGIS Pro map showing the overlap of cropland density (USDA) based on crop variability against California counties in relation to the sampling locations. Average concentration of NP per sample site shown based on circle size given in legend based on GC-MS analysis. Crop variability expressed based on coloration.

Based on Figure 13 above, we can see that Fresno, the sampling site furthest north, is the point nearest an area of high cropland density and agricultural variability. It is also to note that this point is to the east of this area of high agricultural density. It should be subjected to a small amount of onshore northeastern prevailing wind pushing aerosolized pesticides from the

central valley, into this area of study, as shown in figure 14. This supports the hypothesis that there would be a higher concentration of NP in this region.

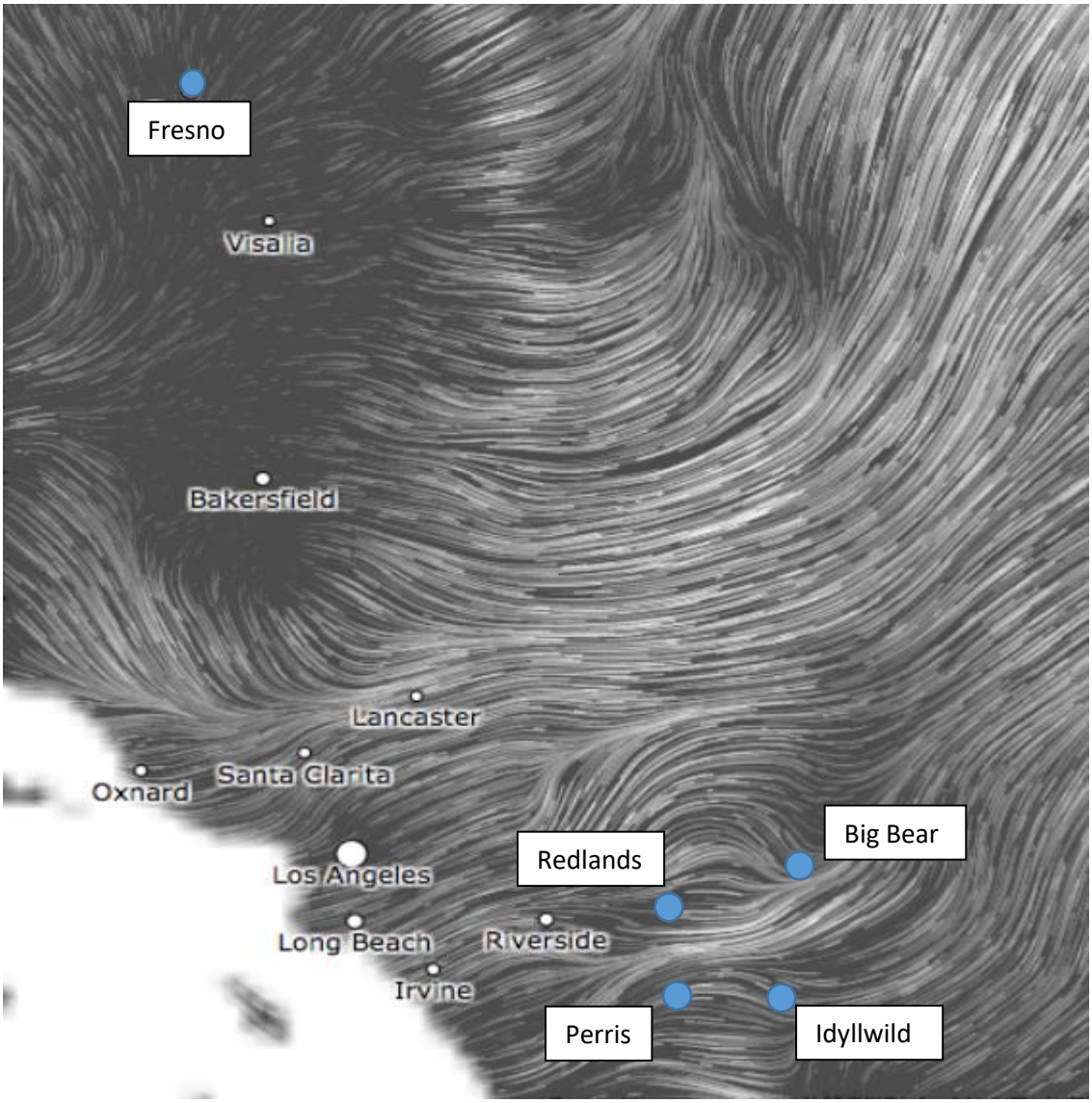


Figure 14: Geographic map indicating wind speed and direction for the southern Californian coast. The direction of the wind is indicated by the path at which the lines move and the speed is dictated by the density of the lines. Figure obtained from: http://hint.fm/wind/.

Additionally, the site of study is also situated on in Fresno county. This area of land is characterized by one of the highest degrees of pesticide usage in all of California, equating to the elevated introduction NPEs into the environment. The quantification of the number of pounds of pesticide per year is shown in the figure 15. The situation of the Fresno

sampling site in this county would only further support the hypothesis that this location would have the greatest concentration of NP out of the 5 sampling locations. This sample location was chosen as a positive control. Although these factors lead to Fresno having a high risk of environmental exposure to NP, this location actually had the lowest concentration of the compound out of any of the sampling locations. This may have been due to the occurrence of an environmental contamination clean up at this sampling location prior to our sampling date. This site was contaminated by a boat fire leading to spillage of nonpolar compounds into the lake. By cleaning these compounds from the lake, concentrations of other nonpolar compounds such as NP may have also been removed from the surface water, leading to the low concentrations measured.

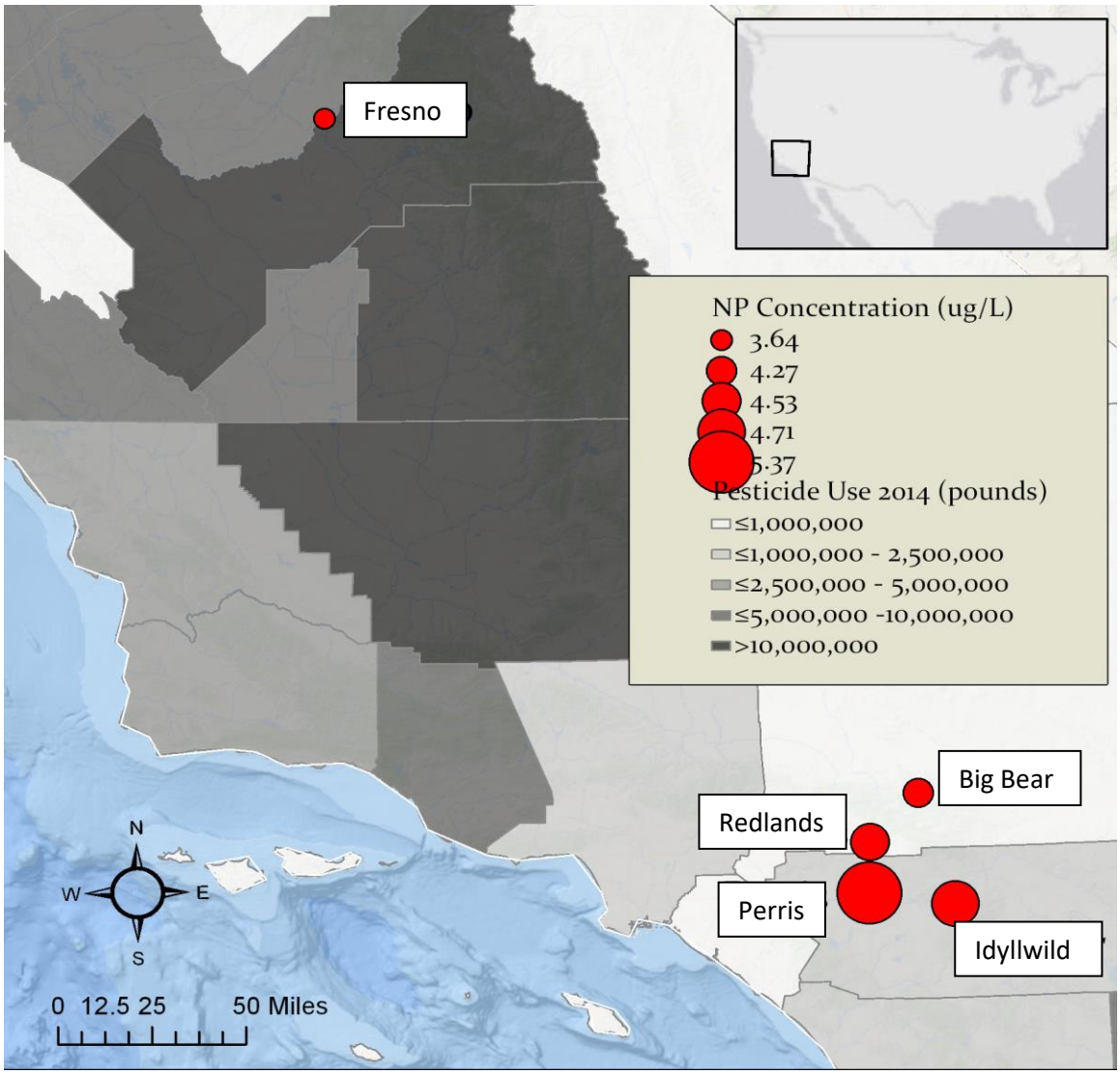


Figure 15: ArcGIS Pro map showing California pesticide usage in pounds based on county in relation to sampling locations. Average concentration of NP per sample site shown based on circle size given in legend based on GC-MS analysis. Pesticide usage per pound shown in legend based on color.

The second location of sampling and the location with the next highest hypothesized NP concentration was Lake Perris, California. This location is in the southern California portion of the state of study. Specifically, it is the most southwest of all the sampling location. This sample point was chosen based on the same criteria as the Fresno point of sampling, distance from an agricultural density center. As seen in figure 13, Perris has a relative amount of agricultural

density however there is not the same variability that we previously saw with Fresno. This area of land is primarily used for raising of cattle and some crops. This lead us to believe that we would see an environmental concentration of NP lower than that of Fresno, yet still relatively high. This sampling site exhibited the highest concentration of NP. We believe saw such a high concentration of NP at this location due to several environmental factors such as: wind direction, geographical structuring of the surrounding area, pesticide usage, and population density. Unlike the northern California sampling location, this point is subjected much more strongly to onshore prevailing winds as seen in figure 14. As earlier stated, this would force the any aerosolized NP inland, allowing for the concentration of the compound on a specific area. When paired with the fact that Perris is in a bowl situated in what is known as a geographical bowl.

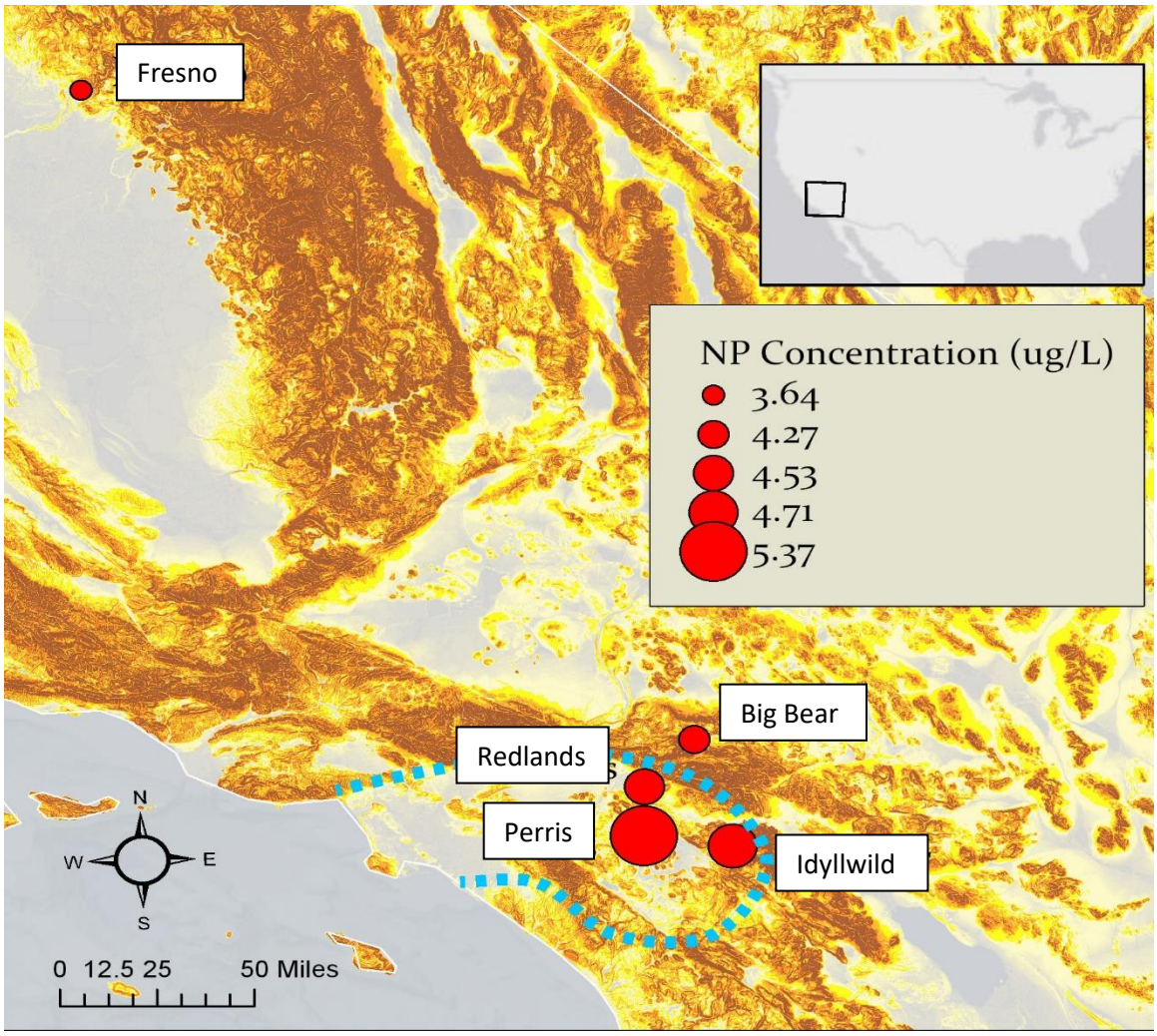


Figure 16: ArcGIS Pro make showing slope and elevation of southern and central Californian mountain ranges with regards sample locations. The degree of slope (0 to 90) is represented by the color gradient seen in the figure. Flat surfaces are represented as gray, shallow slopes are yellow, moderate slopes are orange, and steep slopes are red-brown. Mountainous ridgelines are shown as a drastic change between steep to moderate/low slopes. Geographical bowl outline of southern California show as dotted blue line. Average concentration of NP per sample site shown based on circle size given in legend based on GC-MS analysis.

This geographic bowl shape of inland southern California is shown in figure 16 above as a dotted blue line. The occurrence of the bowl like structure as well as the onshore prevailing winds traps any aerosol or gaseous compounds in its confines, causing its accumulation. This would explain the greater concentration of NP measured at these sampling locations as

opposed to those measured in central California. In this case, Perris would not be subjected to the transport of NP from western sources but instead would be the source of aerosolized NP for more eastern towns. Riverside County contains both the Perris and Big Bear sampling location and has the highest usage of pesticide in pounds per year, as seen in figure 15. This would explain the higher concentrations of NP seen here. Additionally, this location also has a high human population density, which may have influenced the introduction of NPEs and NPs into the environment being that these compounds are used both in commercial and industrial products. The correlation of human population with the presence of environmental NP concentrations can be shown below in figure 17.

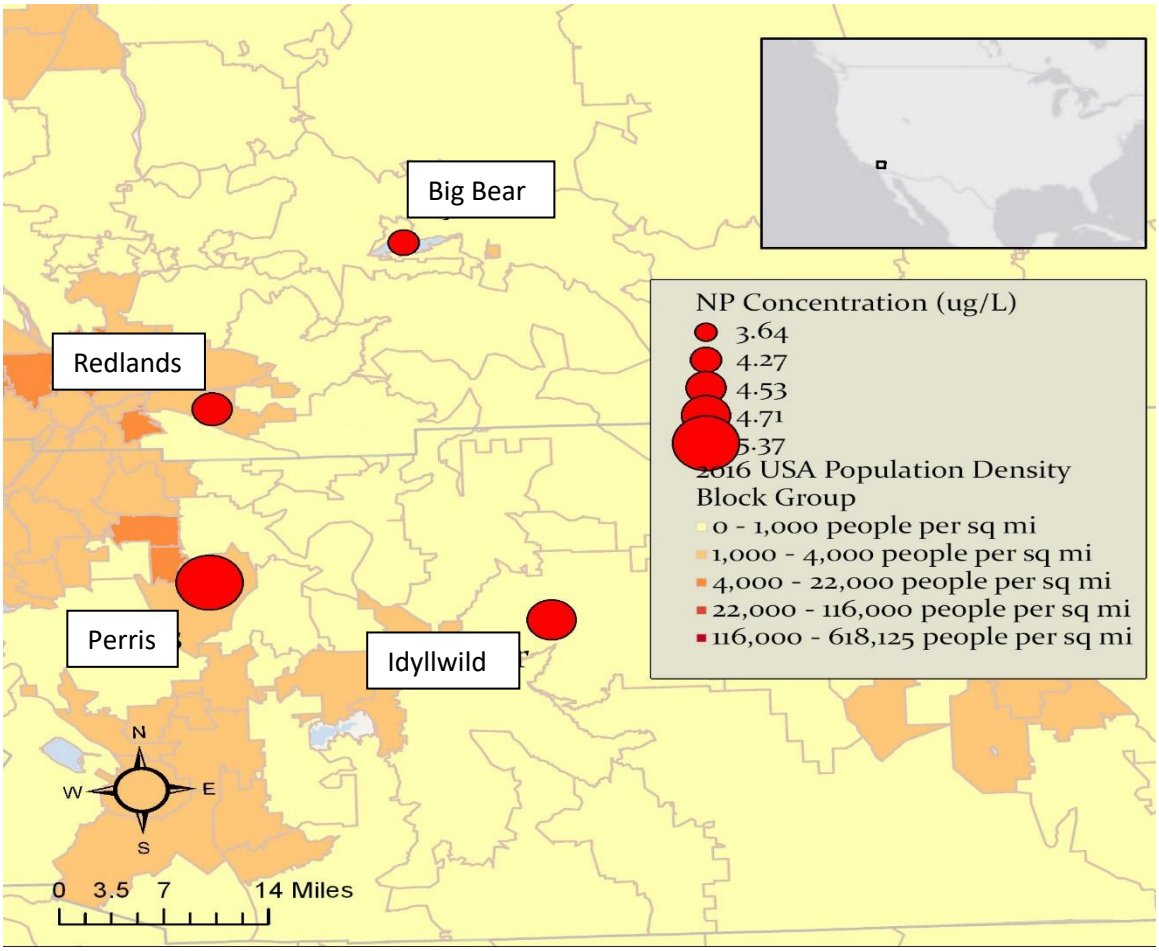


Figure 17: ArcGIS Pro map showing the 2016 population density shown by block area indicated by grey outline in relation to the average concentration of NP measured per sampling location. Population indicated by color. Average concentration of NP per sample site shown based on circle size given in legend based on GC-MS analysis.

The third site of sampling was at the University of Redlands in Redlands, California. This sampling location was chosen for its ease of access as well as its relative distance from an agricultural density centers. At this location, we made use of atmospheric deposition sampling as opposed to the aqueous grab sampling that was used for all other sample locations due to the lack of available surface water at the time of sampling. Although passive atmospheric samples provide a measure of the concentration of NP in the environment, it is used to quantify aerosols in the air compartment rather than in the water compartment. This method of sample collection is dependent on the deposition velocity of the compound of interest and the ability

to measure the sample concentration is dependent on the sample duration. This makes the comparison of water grab samples to passive atmospheric deposition samples difficult if not impossible. One reason for the lower concentration measured at Redlands may be due to the lack of major deposition due to drought, causing there to be a lower accumulation of NP in the sample. Based on figure 13, it is clear to see that Redlands has low agricultural density, little to no crop variability and a significant portion of the land devoted to urban development. The portion of land devoted to urban development was hypothesized not to contribute as much NP into the environment as area of land dedicated to agriculture, however Redlands has a greater risk potential of NP introduction than Big Bear or Idyllwild, being that neither of these locations have agricultural density centers. From GC-MS analysis, this hypothesis was confirmed. We obtain the third highest concentration of NP at this location overall. This sample location was predicted to have a concentration higher than that of Big Bear, but this was not the case. Moderate concentrations of NP in Redlands may be due to similar factors previously stated for Perris, such as: wind direction, geographical structuring of the surrounding area, population density, and most importantly variations in sample collection method.

The fourth location of study was Big Bear Lake in Big Bear, California. This location is marked has little to no agricultural density, seen in figure 13, and is characterized by a mountainous environment. This mountainous environment would potentially provide a source of geographical isolation from any NP transport from more coastal location and the lack of an agricultural density center contributed to the initial hypothesis this this location would have the lowest environmental NP concentration out of the sample locations. Through GC-MS analysis, this was confirmed. The concentration at the Big Bear Lake site was 4.79 μg/L. One possible

factor contributing to the low concentration of NP measured here is the local geography. In figure 15, it can be seen that Big Bear does not fall into the bowl like shape which surrounds the other southern California sample locations. This supports the hypothesis that there is an accumulation of NP within the enclosed bowl region, explaining the lower concentration observed at Big Bear Lake. The second factor possibly contributing to the low concentration at this location may be due to the overall usage of pesticides by the county. As shown in figure 15, Big Bear is situated in San Bernardino County. This county is marked by a low concentration of pesticides used per year. The lower the pesticide usage per year, the lower the concentration of NPEs being introduced into the environment. One final factor contributing to the lower concentration of NP measured could be the smaller population of humans in this area. A lower population would result in a lower availability of manmade products containing NPEs to degrade in the environment.

The last location of study was Lake Fulmor in Idyllwild, California. This location is similar to the Big Bear sampling location as there is little to no agricultural density, as seen in figure 13, and is characterized by a mountainous environment. This location however is closer in proximity to the other sampling locations. Overall, this location was hypothesized to have the second lowest concentration of NP out of the sampling locations due to the presence of the mountainous environment, the lack of an agricultural density center, and the proximity to the sampling site to other agriculturally rich sampling locations. This location was found to have a NP concentration of 4.84 μg/L. The main factors contributing to the moderately low concentration of NP in this area is the wind direction and the local geography. In figure 14, it is apparent that this sampling location experiences a high degree of onshore prevailing wind,

pushing any aerosolized nonylphenol towards the back of the mountainous bowl shown in figure 16. The presence of this high degree of onshore prevailing wind would cause the accumulation of NP in this enclosed area. Since Idyllwild is located on the inner upper rim of the bowl, this sampling location would be susceptible to the deposition of aerosolized NP, however not as prevalently as Perris or Redlands. Other factors which may contribute to the low concentration of NP in this area may also be the low to moderate pesticide usage of the county, seen in figure 15, and the low human population, seen in figure 17.

Representative sampling was obtained as much as possible; however, certain restrictions only allowed limited sampling locations. Geography, and private property are some of such limitations which were considered when choosing sample locations. Specifically, Lake Millerton and Lake Perris were sample sites highly influenced by location limitations, causing sample points to be close together and possibly not entirely representative of the lake itself. The geographic spread of the sampling points however did not adversely affect the deviation of concentrations measured between the sample locations (tables 4-9).

Of the data obtained using GC-MS, there are generally two sample locations with concentrations inconsistent with what was hypothesized. Initially, it was hypothesized that NP concentrations would be the highest at Lake Millerton in Fresno. It was thought that Redlands would display a concentration similar to Big Bear. The reason for the discrepancy between the expected and the actual concentrations may be due to sampling time as well as sampling method. Two weeks prior to the time of sample obtainment at lake Millerton in Fresno, CA, a clean up effort occurred removing various nonpolar compounds from the water. To determine the concentration of atmospheric NP in Redlands, atmospheric deposition samplers were

allowed to collect for a month's time. There is a much lower concentration of NP in the gaseous/aerosol phase than there is in the aqueous phase, and for this reason, the concentration of NP detected in Redlands cannot necessarily be compared to that which was found aqueously.

Conclusion

4-Nonylphenol, a semivolatile organic compound, is capable of causing adverse health effects when present at bioactive concentrations. This compound has many ways of introduction into the environment; however, one of the most prevalent methods of introduction is through the use of non-ionic surfactant containing pesticides. For the collection of environmental samples, both water grab samples and atmospheric passive samples were obtained. GC-MS was used for the detection and quantification of this compound in the environment. The highest concentration of NP in the environment was found in Lake Perris in Perris, California at 5.37 μg/L. This concentration can be explained by environmental conditions such as wind, population density, agricultural density, and the location's presence in a geographical bowl causing the accumulation of the compound in this area. Ultimately, each sampling location, excluding Fresno, followed this trend, being that a greater correlation with the environmental factors led to the greater concentration present at the location of sampling.

The average concentration of NP in the environment was measured at approximately 4.58 ppb, only 2 ppb lower than the threshold for what the EPA describes as safe ambient environmental conditions. NP concentrations were found ubiquitously in the environment; however, it was clear that location as well as many other factors such as local geography, wind

patterns, pesticide usage of an area, recent history clean-up efforts, and general distance from an agricultural density center need to be considered when determining the risk of exposure for an area. From this geographical study, it was found that environmental NP concentrations are generally more quantitively positively correlated with pesticide usage, population, and agricultural density. A correlation regarding wind speed was unable to be determined as most sample locations experienced the same general amount; however, when wind speed is paired with geographical structures like the Southern Californian mountainous bowl, there is a more positive correlation.

The results of this study can be extrapolated to determine not only the risk of exposure for an environment to nonylphenol, but also the population living there. The NP concentration in aqueous environments may give an indication as to the concentration found in other compartments of the environment when taking into account specific partition coefficients. A study of the concentration of NP found in the biotic compartment, would give us an indication as to how this compounds is partitioning from the environment into the human system. The study of how the introduction of these compounds into the environment may be effecting our health as a species is an important area of study being that that adverse health effects have been observed for mammalian NP exposure. To successfully correlate environmental and bodily NP concentrations may ultimately further the understanding of how the deposition of synthetic compounds effects our health and may lead to the enactment of laws limiting the usage of NP and NPE derivatives in agriculture.

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Supplementary:

	Sample Site	Latitude	Longitude
Big Bear - Big Bear Lake	1	34.26128	-116.888208
	2	34.26055	-116.923379
	3	34.25048	-116.962002
	4	34.25147	-116.94166
	5	34.24523	-116.914452
Perris - Lake Perris	1	33.86831	-117.169692
	2	33.85895	-117.175798
	3	33.86508	-117.119751
	4	33.86091	-117.194044
	5	33.87201	-117.163213
Idyllwild - Lake Fulmore	1	33.80455	-116.781158
	2	33.80533	-116.779785
	3	33.80603	-116.778755
	4	33.80488	-116.779527
	5	33.8044	-116.780547
Fresno - Lake Millerton	1	36.99239	-119.683472
	2	36.99733	-119.69463
	3	36.99486	-119.681841
	4	36.99731	-119.678984
	5	37.00188	-119.679499
Redlands - University of Redlands	Anderson Hall	34.06575	-117.166735
	Ann Peppers Hall	34.05915	-117.165198
	Duke Hall	34.06174	-117.1665
	Appleton Hall	34.06412	-117.16141

Figure 1: Culmination of each sampling location and the location of each sampling site given by latitude and longitude coordinates.

GC-MS

Table 2: Concentrations of NP in 1:1 acetone/hexane used to produce a calibration curve. Peak area is in counts and concentrations are given in ppm or mg/L. Produced by Trevor Togashi & Chad Bowyer.

Conc. (ppm)	Peak Area
10	19077
7.5	11622
5	6978
2.5	3092
1.25	1155
0.75	326

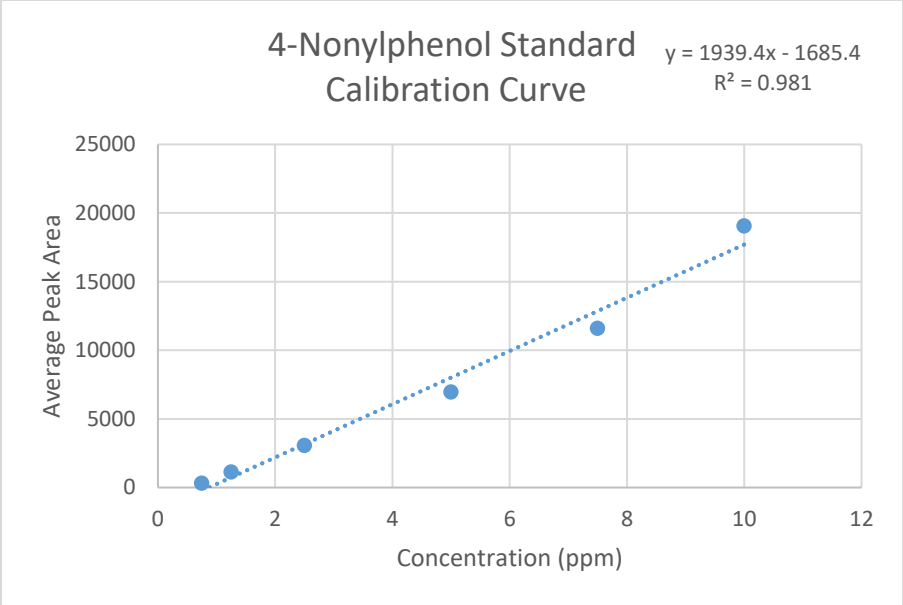

Figure 3: Calibration curve for nonylphenol based on 6 standards. Peak area is in units of counts and the line has a R^2 value of 0.981. Produced by Trevor Togashi & Chad Bowyer. N= 10

Table 4: Concentrations of nonylphenol (GC-MS) in water taken from Big Bear Lake in Big Bear. Concentration are given in parts per billion (µg/L). N=4

Big Bear		
	Concentration NP (µg/L)	
Site 1	6.04	3.68
Site 2	4.35	2.94
Site 3	4.53	5.33
Site 4	3.87	5.69
Site 5	7.73	3.77
	Average	4.79
	STDEV	1.42

Table 5: Concentrations of nonylphenol (GC-MS) in water taken from Lake Millerton in Fresno. Concentration are given in parts per billion (µg/L). N=4

Fresno		
	Concentration NP (µg/L)	
Site 1	4.66	4.28
Site 2	3.52	5.55
Site 3	2.88	2.89
Site 4	2.83	4.52
Site 5	3.46	2.00
	Average	3.66
	STDEV	1.07

Table 6: Concentrations of nonylphenol (GC-MS) in water taken from Lake Fulmor in Idyllwild. Concentration are given in parts per billion (µg/L). The calculated average concentration and standard deviation for the lake excludes all values read as zero/ ND as these samples were non-detectable. Calculated average and standard deviation also exclude measurement of sample two of site one based on grubbs exclusion test. N=4

Idyllwild		
	Concentration NP (µg/L)	
Site 1	4.34	7.14
Site 2	4.37	
Site 3	4.22	4.03
Site 4	4.47	4.18
Site 5	5.93	4.87
	Average	4.84
	STDEV	0.608

Table 7: Grubb's exclusion test results from the site one sample 2. Being that the G-calc value is greater than that of the G-table value, this point is considered an outlier and can be excluded from the calculations of the average concentration and standard deviation. G-table value obtained. [32]

Grubbs table	
Gtable	G-Calc
2.11	2.23

Table 8: Concentrations of nonylphenol (GC-MS) in water taken from Lake Perris in Perris. Concentration are given in parts per billion (µg/L). N=4

Perris		
	Concentration NP (µg/L)	
Site 1	5.14	6.09
Site 2	6.12	6.94
Site 3	4.36	4.48
Site 4	5.02	7.75
Site 5	4.32	3.50
	Average	5.37
	STDEV	1.32

Table 9: Concentrations of nonylphenol (GC-MS) in water taken from Lake Millerton in Fresno. Concentration are given in parts per billion (µg/L). Sample site names correspond to building names around the campus of the University of Redlands. N=4

Redlands		
	Concentration NP (µg/L)	
Appleton	4.391	6.108
Anderson	3.745	3.863
Ann Peppers	3.956	5.130
Duke	4.277	2.316
	Average	4.223
	STDEV	1.1004